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APPLICATION TIMING OF COMPLEX MAGNETIC FIELDS DELINEATES WINDOWS OF POSTTRAINING-PRETESTING VULNERABILITY FOR SPATIAL AND MOTIVATIONAL BEHAVIORS IN RATS*

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Male Wistar rats were trained in a two-session-per-day, non-matching-to-sample radial maze task. During the 2 hr interval between training sessions and testing sessions subjects were exposed for 60 min to either sham fields or to a low intensity (200 to 500 nT) electromagnetic (EM) complex pattern whose electrical (current) equivalent has been shown to elicit long-term potentiation (LTP) in hippocampal slices. This pattern was applied either immediately following the training sessions or immediately antecedent to the testing sessions. Exposures to the experimental field immediately following the training session were associated with an impairment in spatial memory as inferred by increased commissions of reference errors. Exposures immediately prior to the testing session were associated with decreased motivation as inferred by a reduction in speed of responding. These results suggest that the timing of the presentation of patterned magnetic fields may differentially affect the representations of habit strength and drive within the consolidating memory trace.

Keywords: Electromagnetic fields; motivation; spatial memory; rat

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INTRODUCTION

Central nervous system structures that exhibit both endogenous oscillation and synchrony provide target areas for magnetic field coupling (Jenrow, Zhang, Renehan and Liboff, 1998). Structures contained within the electrically labile limbic region, specifically the amygdaloid-hippocampal complex, satisfy these requirements for a physical and functional interaction between neural fields and applied complex magnetic fields. This mesiotemporal aggregate synergistically integrates a developing spatial memory representation with its relevant affective (value) correlate (Edelman, 1989).

Interactions between brain and applied complex magnetic fields should be more probable when the applied magnetic field contains a spatial and temporal structure modelled after biologically generated fields (Persinger, 1997). Long-term potentiation (LTP), as mediated by the hippocampal formation and amygdala, is the putative neural phenomena that facilitates information storage in the mammalian brain (*e.g.*, Kandel *et al.*, 1993). LTP can be elicited in hippocampal slices by the application of a waveform (current) train of burst pulsing embedded in theta rhythm (Rose, Diamond, Pang and Dunwiddie, 1988).

Many learning theories indicate that performance involves both accuracy (habit strength) and motivation (drive). If this concept is applicable within the brain, then magnetic field effects upon memory consolidation could affect both domains. Complex burst-firing magnetic fields elicit behavioral effects consistent pharmacologically with opioid receptor agonism (Fleming, Persinger and Koren, 1994; Thomas, Kavaliers, Prato and Ossenkopp, 1998). The present experiment was designed to differentiate spatial contributions from affective contributions to the developing engram, essentially a dissociation of habit strength from motivational variables.

Interference with the spatial ("habit strength") component of performance should be manifested behaviorally as an increased commission of reference errors, most conspicuously when the complex fields are applied immediately after learning (Squire, 1986). Furthermore, effects on spatial memory systems should attenuate over time concomitant with known shifts in brain regions responsible for encoding spatial representations. As opioid agonistic-like effects

subsequent to complex magnetic fields attenuate quickly (Thomas *et al.*, 1998), relative motivational declines should be most evident for field application immediately before testing.

MATERIALS AND METHODS

Twenty eight male Wistar albino rats served as subjects in two experiments. Animals were obtained from Charles River (Quebec) and were housed in groups of three per cage in standard colony housing. Ambient temperature was maintained between 20 and 21°C with a 12:12 light-dark cycle (photophase onset at 07:30 h). Food and water were available to the animals *ad libitum*.

The experiments were procedurally similar except for age and the food deprivation schedule. Subjects in the first and second study were approximately 7 and 4.5 months old, respectively. Food deprivation in the first study was accomplished by housing the rats ($N = 12$) in single cages and slowly reducing them to 80% of their free-feeding body weights. Rats in the second study ($N = 16$) remained group housed and were placed on a 23-hour per day food deprivation schedule.

Animals were pretrained for ten sequential days in a two-session-per-day non-matching-to-sample eight-arm radial maze paradigm. During the first daily (training) session all eight arms were associated with reward. Animals could enter any four arms to obtain reinforcement (two 50 mg Noyes food pellets). They were removed immediately before entering any of the remaining four arms. During the second daily (testing) session (completed 2 hr later) animals were rewarded only for entering the four arms not rewarded during the training session.

During the 2 hr inter-session interval subjects were relocated to the room housing the equipment used to generate the patterned magnetic fields. Details of the equipment used in this study have been described elsewhere (Stewart and Persinger, 1999). During the ten pretraining days the computer that was used to generate the patterns was activated but disconnected from the equipment which emitted the fields.

Ten days of treatment followed the pretraining. Animals received whole body exposure to the patterned magnetic field (simulating hippocampal theta rhythm and burst firing) for 1 hr either immediately

following completion of the training session ($N = 11$) or for 1 hr following a 1 hr delay ($N = 11$). A control group ($N = 6$) was placed in the treatment room in a location where the magnetic field intensity from the extremely low frequency magnetic fields (1 – 100 Hz) was below measurable levels (< 10 nT). Each presentation of the pattern was defined as one stimulus and the stimulus sequence was presented for 60 min at one of five different daily inter-stimulus intervals (0 msec, 1 msec, 2000 msec, 4000 msec and 10000 msec on days 1 through 5, respectively). The same sequential order of inter-stimulus intervals were employed in both blocks of testing. Similar inter-stimulus intervals have been successfully employed in previous research (Persinger, in press). Moreover, this daily variation satisfies the theoretical requirements of the resonance model for non-habituation to the magnetic fields. Placement of the rat proximal to the edge of the apparatus or placement of the rat in the center of the apparatus resulted in exposures to magnetic fields with intensities (B) reaching maxima of either 500 or 200 nT, respectively.

The number of reference errors (defined as entering an arm during the testing session that had been rewarded during the training session), total number of arms entered, and total time to complete the maze were employed as indicators of performance. All analyses were completed on a VAX 4000 computer using SPSS software. MANOVA (repeated measure) was the primary model of analysis. Partial eta-squared values were obtained to estimate the effect size (amount of variance explained) for all statistically significant effects.

RESULTS

Four-way analyses of variance with two levels repeated (two blocks of five inter-stimulus intervals per block) and two levels not repeated immediate post-training exposure, delayed post-training exposure and sham field; experiment 1 *versus* experiment 2 revealed no statistically significant differences between experiments for any of the dependent measures [$F(1, 18) \leq 4.0$, $p > .05$]. Two-way interactions between experiment and treatment and experiment and block were not statistically significant. Consequently the data from the two experiments were combined to facilitate the analyses.

There was a significant treatment main effect for total time required to complete the testing session [$F(2, 25) = 14.08$, $p < .001$, $\varepsilon^2 = 0.53$]. *Post hoc* analysis indicated the source of this effect was due to the longer times displayed by the group that received the magnetic fields one hour after daily training (1 hr before testing) compared to the groups that received the sham field or the magnetic fields immediately after training. The group that received the delayed treatment required about *twice* the amount of time to complete daily testing in the maze relative to the sham field group.

A main effect for block [$F(1, 25) = 37.06$, $p < .001$, $\varepsilon^2 = 0.60$] was noted and *post hoc* analysis indicated this was due to the decrease in maze completion times during the second block. A statistically significant interaction between blocks of field presentation and treatment was also apparent [$F(2, 25) = 4.40$, $p < .05$, $\varepsilon^2 = 0.26$]. *Post hoc* analysis indicated that the primary source of the interaction was due to the longer times required to complete the maze for the group exposed immediately to the magnetic fields relative to the sham field group for the first but not the second block of trials. The means and standard errors of the means are shown for each treatment in each block in Table I.

There were no statistically significant treatment effects for the total number of arms entered [$F(2, 25) = 0.29$, $p > .05$]. Therefore increased maze completion times for the subjects receiving delayed field treatment occurred without a concomitant increase in number of arms entered (a motivational inference). A block effect was noted for

TABLE I Means and standard errors of the mean (SEM) for average time to complete the testing session per block for rats exposed to sham conditions or to patterned magnetic fields for 60 min either immediately following the training session or after a 60 min delay

Block	*Field presentation*	*Mean (sec/day)*	*SEM*
1*	Immediate	81.6[a]	4.42
	Delayed	108.7[b]	8.40
	Control	56.7[c]	4.42
2**	Immediate	58.8[a]	2.66
	Delayed	79.1[b]	5.66
	Control	51.2[a]	4.21

*[a] *vs.* [b] *vs.* and *vs.* [c] *post hocs* $p < .01$.
**[a] *vs.* [b] *post hocs* $p < .01$.

total number of arms entered [$F(1, 18) = 52.43$, $p < .001$, $\varepsilon^2 = 0.74$]. *Post hoc* analysis revealed that the total arms entered decreased from the first block to the second block.

Ten days of pretraining in this radial maze task was associated with an asymptote in the commission of reference errors that approached the ceiling limits of the measure. Discerning group differences therefore required extracting within subject variability from the variance associated with differences between individuals by employing each animal as its own control. This was completed by dividing daily reference errors (incurred during the treatment blocks) by the mean number of reference errors committed during the last three days of pretraining.

A main effect for relative change in the numbers of commission errors for reference memory was noted for the second block [$F(1, 20) = 5.50$, $p < .05$, $\varepsilon^2 = 0.22$] as a function of treatment. This effect was not statistically significant for the first block [$F(1, 20) = 3.00$, $p = .10$]. *Post hoc* analyses discerned the source of the effect in the second block was due to increased commission of reference errors when the magnetic field was applied immediately following the training session relative to the group receiving a 1 hr delay before field application (Tab. II).

Results are presented relative to 1.0, where 1.0 denotes equivalence to the performance asymptote established during pretraining, values greater than 1.0 reflect relative performance impairments and values less than 1.0 reflect relative performance improvements. These relative

TABLE II Means and standard errors of the mean (SEM) for average relative reference error commissions per block for rats exposed to sham conditions or to patterned magnetic fields for 60 min either immediately following the training session or after a 60 min delay

Block	*Field presentation*	*Mean (err/day)*	*SEM*
1	Immediate	1.07[a]	0.16
	Delayed	0.74	0.09
	Control	0.60[b]	0.09
2	Immediate	0.65[c]	0.13
	Delayed	0.31[d]	0.07
	Control	0.44	0.13

[a] *vs.* [b] *post hoc* $p = .05$ and [c] *vs.* [d] *post hoc* $p < .05$.

data correspond to actual mean (±SEM) daily reference errors of 1.80(0.13), 1.76(0.17) and 1.50(0.23) during the first block and 1.04(0.18), 0.82(0.19) and 1.07(0.27) during the second block for immediate, delayed, and sham field application respectively. Additional MANOVAS were completed to assess the contribution of magnetic field intensity (B) on the dependent measures. There were no statistically significant findings attributable to intensity.

DISCUSSION

Applications of weak magnetic pulses with putative LTP-evoking properties for 60 min immediately following the training session were associated with increased numbers of reference errors, regardless of the interval (0 msec, 1 msec, 2000 msec, 4000 msec, or 10000 msec) between pulses. Application of the same waveform for 60 min immediately before the testing session did not affect the numbers of errors but was associated with decreased rates of responding (longer maze completion times but equivalent numbers of total arm entries). Thus a dissociation between the spatial (reference error) component and affective (motivational) component of memory may have occurred.

Presentations of patterned magnetic fields immediately prior to testing resulted in a decrease in the rate at which this appetitively motivated task was performed. As protracted food deprivation in nature would ultimately constitute a stressful condition, this motivational change may have been a function of augmented antinociception. Modulation of antinociception has previously been demonstrated by application of patterned magnetic fields whose actions were blocked by opioid receptor antagonism (Thomas *et al.*, 1998).

Applications of pulsed magnetic fields during the period immediately following training were associated with greater numbers of reference errors than when exposures were delayed by one hour. This finding is consistent with the vulnerability of the memory trace during the initial consolidation stage following a learning event (Squire, 1986). Errors following immediate field applications were statistically significantly different from delayed field applications during the second block but not the first block. This result suggests that the "window of vulnerability" to the effects of this field pattern changed

over time and practice. Recent reports (*e.g.*, Van Horn *et al.*, 1998) have shown that throughout protracted task repetition there were shifts in metabolic activity to different areas of the limbic system.

This study has reported the dissociability of optimal windows of exposure for interference with the spatial and motivational components of a consolidating memory trace. However, in a more naturalistic setting replete with perpetually changing task parameters these windows would overlap and exist continuously. Moreover, the electromagnetic stimuli necessary to intercalate with these processes may exist ubiquitously in nature (see Persinger, Ludwig and Ossenkopp, 1973), complemented by fields from electromagnetogenic devices. The effects of the fields in this study accommodated about 50% of the variance in time to complete the task and 20% of the variance in commission of errors. These robust effect sizes were obtained in a population of normal subjects rather than subjects with experimentally induced limbic lability. This suggests that weak intensity magnetic stimuli may effect the population as a whole during normal neuronal lability following the acquisition of novel information.

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